

# Nonalcoholic Fatty Liver Disease and Cardiovascular Disease Risk

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Nonalcoholic fatty liver disease (NAFLD) is prevalent in people with the metabolic syndrome and type 2 diabetes. Evidence is now accumulating that NAFLD is associated with obesity and diabetes and may serve as a predictor of cardiovascular disease. Although at present, treatment of the individual risk factors pertinent to NAFLD is advocated, novel therapies are emerging that may target steatosis and/or inflammation, thus ameliorating the overall cardiovascular disease risk. Long-term outcome studies need to establish whether treatment of NAFLD (and in particular which therapy) will affect the long-term outcome.

## Introduction

In the past decade, nonalcoholic fatty liver disease (NAFLD) has gained much interest. NAFLD, which is characterized by a wide spectrum of liver disease ranging from liver steatosis to the more severe nonalcoholic steatohepatitis (NASH), resembles alcohol-induced liver disease. By definition, NAFLD develops in subjects who are not heavy alcohol consumers and who have negative tests for viral and autoimmune liver diseases. It usually has a benign clinical course, but it may progress to NASH, fibrosis, cirrhosis, and rarely to hepatocellular carcinoma [1,2]. The term NASH was introduced by Ludwig et al. [3] in 1980, who reported 20 moderately obese patients with liver biopsy changes resembling alcohol-induced hepatitis, although none of these patients reported a history of alcohol abuse. In recent years, NAFLD has gained appreciation as a pathogenic factor of insulin resistance and type 2 diabetes mellitus (T2DM). Furthermore, several studies showed an association between NAFLD and features of the metabolic syndrome, including dyslipidemia and (visceral) obesity, stressing the association with insulin resistance

as an important feature of NAFLD. Currently, NAFLD is considered by some authors to be the hepatic component of the metabolic syndrome [4,5] and evidence is accumulating that patients with NAFLD have an increased risk for developing cardiovascular disease (CVD).

## Epidemiology of NAFLD

NAFLD is a common condition and may be the most prevalent liver disorder; however, the true incidence and prevalence in the general population are not known. To date, the prevalence of NAFLD has been estimated in selected patient populations or in studies that are to some extent population based. In the NHANES III (third National Health and Nutrition Examination Survey), a population-based sample of over 15,000 subjects, the prevalence using abnormal (ie, above the upper limit of “normal”) values of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) as markers of NAFLD was estimated to be 7.8%, of which 31% had an identifiable cause, which would yield a prevalence of NAFLD of 5.4%. However, the reported prevalences are heavily dependent on the chosen cutoff points for ALT. A study in Northern Italy using ultrasound identified fatty liver in 16% of lean nondrinkers and a prevalence of 76% in obese nondrinkers [6]. An ultrasound screening study in a Japanese general population found a prevalence of 12%; however, this study did not exclude heavy drinkers, which might have overestimated the prevalence of NAFLD [7]. Overall, in the general population, the estimates of NAFLD range between 3% to 36%, but with most estimates in the lower range. In obese patients and in patients with T2DM, the prevalence of NAFLD is much higher and may be up to 90% [8]. However, comparisons of the reported prevalences of NAFLD are largely hampered by the use of different definitions in greatly differing study populations.

## Diagnosis of NAFLD

The majority of patients with NAFLD are asymptomatic, but some may complain of fatigue and right upper quadrant abdominal fullness or even pain. Laboratory analyses performed in patients with complaints or at health checkups show moderately elevated levels of ALT and AST, with an

AST-to-ALT ratio usually less than 1. The other enzymes,  $\gamma$ -glutamyl transferase and alkaline phosphatase, may also be elevated. Noninvasive imaging techniques, such as ultrasonography and CT, may assist in the diagnosis of fatty liver disease. Ultrasound examination of the liver has a sensitivity and specificity of 80% to 90% in identifying fatty liver infiltrates, compared with liver biopsies [9]. CT has a higher sensitivity, but is more expensive than ultrasonography. In contrast to liver biopsies, these imaging techniques cannot distinguish steatosis from steatohepatitis. However, the role of liver biopsy, which is regarded as the “gold standard,” is controversial because of the elaborate and invasive nature and associated risks of this technique, and the general benign clinical course of NAFLD. In addition, no consensus exists on the standardization of diagnostic criteria based on liver histology, although a number of scoring systems for NAFLD have been introduced [10••]. Only patients who are suspected to have an advanced stage of the disease, based on clinical judgment, ultrasound, and biochemistry, should be considered for liver biopsy. For example, in a patient with T2DM, morbid obesity, advanced age, or an AST-to-ALT ratio greater than 1, a biopsy may be considered. [11]. The American Gastroenterological Association (AGA) recommends that in patients with suspected NAFLD a detailed history on alcohol intake should be taken to exclude the possibility of alcohol-induced hepatic steatosis and steatohepatitis. The initial laboratory assessment should include ALT, AST, alkaline phosphatase, serum bilirubin and albumin levels, prothrombin time, and serologic tests for viral hepatitis infection. An imaging study should be performed when other causes of liver disease have been excluded. The decision to perform a liver biopsy may depend on the specific clinical circumstances of individual patients in addition to the risks and benefits of performing a liver biopsy and should always, as stated by the AGA, include the patient in the decision making process [12].

In epidemiologic studies, ALT has been used as a marker for liver fat accumulation. Studies assessing the specificity and sensitivity of ALT as a marker of NAFLD are limited [13,14] and applying the laboratory defined cut-off value (ie, upper limit of the laboratory reference range) may underestimate the prevalence of NAFLD [14]. Prati et al. [15] have suggested new cutoff values for ALT in order to facilitate the identification of subjects with NAFLD. However, because cutoff values largely depend on the assay used, this proposal has been questioned [16]. One study has assessed the correlation of ALT with proton spectroscopy MRI; it found a modest, but significant correlation ( $r = 0.5$ ) [17]. As is the case for clinical diagnosis, a detailed history of alcohol intake is mandatory to exclude those patients with ALT elevation caused by alcohol abuse and to address alcohol intake as a possible confounder or effect modifier. Viral and autoimmune liver disease and hepatotoxic medication should be considered as well. With these limitations in mind, ALT may be an acceptable marker for hepatic steatosis in epidemiologic studies.

## NAFLD in Relation to the Metabolic Syndrome and Type 2 Diabetes

The putative role of the liver in the pathogenesis of T2DM has gained much interest. Several cross-sectional studies have demonstrated that NAFLD is related to features of the metabolic syndrome and T2DM [18–20]. In the analysis of the NHANES III, up to 31% of the elevated aminotransferase activity could be explained by high alcohol consumption, hepatitis B or C infection, and/or high transferrin saturation, whereas in the remaining 69%, the elevated ALT activity was significantly associated with higher body mass index (BMI), waist circumference, triglycerides, fasting insulin, and lower high-density lipoprotein (HDL) cholesterol [18]. Several studies have addressed the prospective relation of ALT and the metabolic syndrome and T2DM. Nakanishi et al. [21] found that ALT was associated with future risk of metabolic syndrome in middle-aged Japanese men, but used BMI instead of waist circumference to define the metabolic syndrome. Hanley et al. [22] studied the relationship of four different liver enzymes (including ALT) with the development of the metabolic syndrome in a multi-ethnic cohort. They demonstrated that ALT was associated with an increased risk of incident metabolic syndrome. In the Hoorn Study, a population-based cohort study among elderly white men and women, we found that ALT was associated with the development of the metabolic syndrome after 6 years of follow-up [23].

In patients with T2DM, elevated serum ALT enzyme activity is more frequently observed than in the general population [24,25]. In addition, some [26–30], but not all studies [21,31–33] have demonstrated independent and significant associations of ALT with future T2DM. In 1988, Ohlson et al. [26] demonstrated that baseline ALT was a predictor of incident T2DM after 13.5 years of follow-up in a cohort of 766 Swedish men, with a significant fourfold risk for those men in the upper quintile compared with those in the lower quintile. In the final multivariate model, ALT was a predictor of incident T2DM in 451 Pima Indians after an average of 6.9 years, after adjustment for fasting blood glucose levels, BMI, bilirubin, systolic blood pressure, uric acid, and a family history of diabetes [26]. Vozarova et al. [27] found that higher ALT (upper compared with lower decile) was a significant and independent predictor of T2DM with a twofold increase risk, after adjustment for age, sex, body fat, insulin sensitivity, and acute insulin response. Other studies, mainly performed in men, confirmed these earlier studies [28,29], whereas recent population-based studies could not demonstrate independent associations of ALT and future risk of T2DM [32,33]. The observed association between ALT and incident T2DM in the mentioned studies may be explained by the fact that they were performed in high-risk populations, which may not be representative of the general population. Overall, these studies show that patients with NAFLD are at increased risk for T2DM and the metabolic syndrome, suggesting that the increased CVD risk may be mediated via components of the metabolic

**Table 1. Cardiometabolic risk determinants or surrogate end-point markers associated with NAFLD and NASH**

Study	CVD risk determinants
Nakanishi et al. [21], Hanley et al. [22], and Schindhelm et al. [23]	Metabolic syndrome
Ohlson et al. [26], Vozarova et al. [27], Sattar et al. [28], Hanley et al. [29], and Wannamethee et al. [30]	Type 2 diabetes mellitus
Brea et al. [34] and Targher et al. [35,36]	Carotid intima-media thickness
Schindhelm et al. [38] and Villanova et al. [39]	Endothelial dysfunction
Kerner et al. [45] and Targher et al. [47]	Oxidative stress and inflammation
Pagano et al. [48] and Targher et al. [49]	Adiponectin
Pagano et al. [52]	Resistin
Cassader et al. [54] and Musso et al. [55]	Postprandial hypertriglyceridemia
Su et al. [56]	Postprandial hyperglycemia
CVD—cardiovascular disease; NAFLD—nonalcoholic fatty liver disease; NASH—nonalcoholic steatohepatitis.	

syndrome and T2DM. Because the pathophysiology linking NAFLD with either the metabolic syndrome and/or diabetes has not been clarified, it is difficult to make the distinction between confounding and mediating variables in the epidemiologic analyses.

### NAFLD and Increased CVD Risk

Several cross-sectional studies have demonstrated an increase in carotid artery intima-media thickness (CIMT) in patients with NAFLD [34–36]. However, in these studies diagnosis of NAFLD was based on evaluation of liver enzymes or on ultrasound evaluation but not confirmed by liver biopsy, which is regarded as the “gold standard” in the diagnosis of NAFLD [10••]. In a recent study, Targher et al. [37••] demonstrated that patients with biopsy-proven NAFLD have a significantly higher CIMT compared with age-, sex-, and BMI-matched healthy control subjects. Moreover, they demonstrated that the histologically assessed NAFLD predicted CIMT, independent of classical risk factors, including insulin resistance and components of the metabolic syndrome.

Previously, in patients with well-controlled T2DM, we have shown that slightly elevated ALT, as a surrogate marker of NAFLD, is associated with a decreased brachial artery flow-mediated vasodilatation and an impaired whole-body insulin sensitivity [38]. In line with the observation in patients with T2DM, it was shown that nondiabetic patients with NAFLD have a decreased brachial artery flow-mediated vasodilatation compared with matched healthy control subjects, independent of other risk factors, including insulin resistance and components of the metabolic syndrome [39]. Ioannou et al. [40] studied the association of ALT and the calculated 10-year risk of coronary heart disease as estimated with the Framingham risk score. Subjects with elevated ALT values had a significantly higher Framingham risk score compared to those with normal ALT values, indicating a higher CVD risk in patients with NAFLD [40].

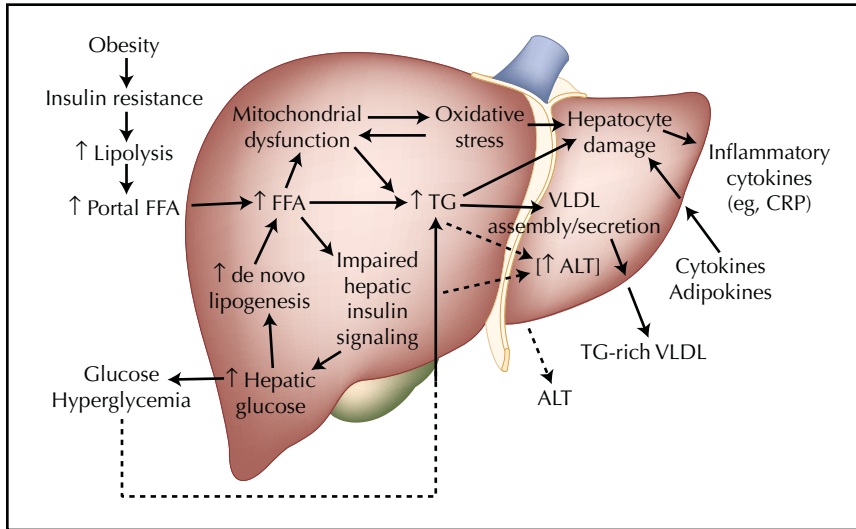
Only a limited number of studies have addressed the prospective relation of ALT with CVD and mortality. In the Hoorn Study, a population-based cohort of white men and women aged 50 to 75 years, we studied the association of ALT at baseline with all-cause mortality and incident cardiovascular and coronary heart disease events [41] and found a significant association of ALT with coronary heart disease after adjustment for components of the metabolic syndrome and traditional CVD risk factors. We found no independent associations of ALT with CVD events and all-cause mortality. The latter result was in line with a previous study by Arndt et al. [42], who studied the association of ALT with all-cause mortality in 8043 male construction workers and found no significant association. In contrast, a recent study by Nakamura et al. [43] found a positive association of ALT with all-cause mortality in Japanese men and women, but only for those with a BMI below the median (22.7 kg/m<sup>2</sup>).

### Mechanisms Linking NAFLD to Increased CVD Risk

The increased CVD risk associated with NAFLD might be explained by the close relation of NAFLD with components of the metabolic syndrome and T2DM; however, recent studies support the notion that NAFLD in itself might contribute to the increased CVD risk (Table 1). However, the mechanisms for this putative relationship are not clear. Several probably highly interrelated factors contribute to the enhanced risk of diabetes and metabolic syndrome in persons with NAFLD (Fig. 1).

#### Oxidative stress and inflammation

Hepatic steatosis mediated by insulin resistance is required for subsequent events (of which oxidative stress is an important contributor) that lead to liver injury and disease progression [44]. However, the underlying factors that promote disease progression to cirrhosis are not understood. Enhanced free fatty acid oxidation increases the formation of oxygen



**Figure 1.** Overview of the mechanisms—increased uptake of free fatty acids (FFAs) by the liver, an increase in de novo lipogenesis, impaired  $\beta$  oxidation caused by mitochondrial dysfunction, and an insufficient assembly and secretion of very low density lipoproteins (VLDLs)—involved in the development of nonalcoholic fatty liver disease (NAFLD) and the contribution of NAFLD to cardiovascular risk. ALT—alanine aminotransferase; CRP—C-reactive protein; TG—triglycerides.

radicals, in turn leading to lipid peroxidation, mitochondrial dysfunction, and cell damage with subsequent release of cytokines, including tumor necrosis factor- $\alpha$ , interleukin-6, and C-reactive protein (CRP). Kerner et al. [45] found an association of ALT with CRP. In contrast, Haukeland et al. [46] studied the role of systemic inflammation in individuals with NAFLD and NASH compared with healthy control subjects. It was demonstrated that interleukin-6, but not CRP, after adjustment for BMI, age, and sex, was elevated in NAFLD compared with control subjects and tumor necrosis factor- $\alpha$  was higher in NASH compared with NAFLD [46]. Of interest, a study by Targher et al. [47] demonstrated that CRP was elevated in individuals with NAFLD, but this association was largely explained by the amount of visceral fat.

#### Adiponectin, leptin, and resistin

Decreased adiponectin levels may represent another mechanism linking NAFLD to CVD. Patients with NAFLD have lower levels of adiponectin compared with healthy control subjects, independent of components of the metabolic syndrome [46,48,49]. This observation may be relevant because some studies have shown that lower levels of adiponectin are associated with CVD [50]. Leptin is a cytokine hormone mainly produced by adipocytes, which regulates food intake and fat metabolism through actions on the central nervous system. It has been suggested that leptin may act as one of the regulators in progression of NAFLD to NASH by upregulation of transforming growth factor- $\beta$  [51]. However, a recent study found no association of leptin with liver disease severity [52]. Resistin is a protein expressed in adipose tissue and related to insulin resistance in mice [53]. Recently, a relationship of resistin, NAFLD, and NASH was demonstrated in humans, with higher plasma resistin levels in individuals with NASH [52].

#### Postprandial dysmetabolism

Studies comparing the postprandial response of triglycerides and free fatty acids to a fat-rich meal in nondiabetic

subjects with biopsy-proven NASH to control subjects showed that patients with NASH had significantly higher postprandial triglyceride levels than healthy control subjects [54,55]. Another study demonstrated that patients with ultrasound-diagnosed NAFLD with an abnormal ALT and/or AST had higher glucose levels after a 75-g oral glucose tolerance test than those with normal ALT and/or AST [56]. Toledo et al. [57••] observed that in obese patients with T2DM, increased hepatic steatosis, as quantified by CT scanning, was positively correlated with serum triglycerides and inversely with HDL cholesterol. No difference in low-density lipoprotein (LDL) cholesterol and apolipoprotein B100 was observed relative to the degree of hepatic steatosis. However, the LDL particle size was smaller in the group with severe hepatic steatosis. These observations indicate that hepatic steatosis may contribute to the increased CVD risk in these patients by increasing triglyceride enrichment of very low density lipoprotein (VLDL) particles, lowering HDL cholesterol and by increasing small, dense LDL particles. The relationship of hepatic steatosis with serum triglycerides was stronger in subjects with a minor degree of hepatic steatosis and weaker in those with a more severe degree of hepatic steatosis, which may, as the authors suggested, indicate that the incorporation of triglycerides into VLDL may have a limited capacity [57••].

#### Management of NAFLD-associated Cardiometabolic Abnormalities or NAFLD?

Lifestyle interventions, including diets and exercise, leading to weight loss and concomitant improvement of the CVD risk profile, have been shown to lower liver fat content [58,59]. These interventions may improve insulin sensitivity and/or reduce oxidative stress, both important pathophysiologic factors in the pathogenesis of NAFLD and CVD. Although these lifestyle interventions seem promising in the short term, long-term effects have not yet

been established and might not be sufficient in patients with multiple cardiometabolic risk factors, such as persons with the metabolic syndrome and/or T2DM.

Several pharmacologic agents, including metformin and peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) agonists, have been used in the treatment of NAFLD. An open-labeled trial of 20 mg/kg of metformin for 1 year demonstrated only a transient improvement of liver enzymes [60]. Tiikkainen et al. [61] compared the effect of 2 g of metformin to 8 mg of the PPAR- $\gamma$  agonist rosiglitazone on liver fat content and hepatic insulin sensitivity in a 16-week double-blind randomized trial in 20 subjects. They found that rosiglitazone decreased liver fat by 51%, whereas no significant decrease was observed in the metformin-treated subjects. In contrast, Bugianesi et al. [62] found that 2 g of metformin led to more ALT normalization (ie, below the reference range) than either vitamin E or diet-induced weight reduction in 55 subjects in a 12-month open-labeled randomized trial. Metformin induced a decrease of 50% in liver fat and a decrease of inflammation and necrosis, using paired liver biopsies [62]. Unfortunately, in this study no paired biopsies were performed in the subjects who were randomized to vitamin E or the weight reduction intervention; thus, the level of liver histology improvement in the treatment group could not be compared with the effect in the control group. A recently reported 6-month placebo-controlled trial of diet combined with pioglitazone compared to diet with placebo in 55 patients with liver biopsy-proven NAFLD demonstrated a significant decrease in ALT and hepatic fat content (by 54%) and a significant increase in hepatic insulin sensitivity [63••].

More recently introduced pharmacologic agents, including glucagon-like peptide-1 (GLP-1), receptor agonists, incretin-mimetics known to promote insulin production and secretion, and rimonabant, a cannabinoid receptor-1 (CB1) antagonist, may be of therapeutic value in the treatment of NAFLD and NASH. In ob/ob mice, Ding et al. [64] demonstrated that 60 days of administration of a GLP-1 receptor agonist reduced hepatic steatosis. This reduction in hepatic fat content was not only related to a decrease in body weight, but also to a decrease in regulatory genes of fatty acid synthesis [65]. Recently, Tushuizen et al. [65] reported on a 59-year-old male with T2DM treated with a GLP-1 receptor agonist for 44 weeks. They found a significant decrease in liver fat measured by liver spectroscopy, suggesting that GLP-1 receptor agonists may be a potential treatment modality in patients with NAFLD. However, more studies assessing the effect of GLP-1 receptor agonists on hepatic steatosis are needed to confirm these results and assess the applicability of these agents for the treatment of hepatic steatosis [65]. Rimonabant has been shown to be effective in weight reduction and improvement of lipid profile [66]; however, the effect of rimonabant on lowering of liver fat content and improvement of liver histology beyond weight loss in

patients with NAFLD needs to be evaluated. Of interest, a recent study demonstrated that treatment with the CB1 receptor antagonist SR14716A in mice inhibited fibrosis in liver injury models [67], suggesting that treatment with a CB1 receptor antagonist may be a future therapeutic modality in NAFLD and NASH.

## Conclusions

Evidence is now accumulating that NAFLD is associated with cardiovascular risk factors, with markers of subclinical atherosclerosis, and with overt CVD events. This increased risk for CVD necessitates the evaluation and treatment of these patients. Long-term outcome studies need to establish the benefit of treatment of NAFLD on the reduction and prevention of diabetes and CVD risk.

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